with lipid bilayers are described and understood quite well. Recently, new types of detergents with cyclohexyl groups or branches in their hydrophobic tails have been synthesized and proposed to be superior for membrane protein studies. Cymal-6 has, for example, been used for isolating membrane proteins such as CCR5 and HIV-1 corepressors. Here we provide a rather comprehensive description of the interactions of Cymal-6 with fluid membranes of POPC. This includes the temperature-dependent phase behavior (i.e., the onset and completion of solubilization), membrane partitioning, disordering, and permeabilization as seen using ITC, time-resolved fluorescence anisotropy of DPH, dynamic light scattering, and the lifetime-based vesicle leakage assay.

421-Pos Board B190
Characterizing the Interactions of Lysophospholipids with Lipid Membranes
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This study aims to characterize and compare thermodynamic interactions of the lysophospholipid - C12 - lysophosphocholine (lysoPC) and its synthetic analog, n-dodecylphosphocholine (DPC) - with lipid membranes. As a biomolecule possessing detergent-like properties, lysoPC is involved in many biological processes and DPC has been used widely in NMR studies of membrane proteins. We investigate the lipid-detergent systems by determining partition coefficient, mole ratios of bound detergent to lipid at membrane saturation and solubilization boundaries, and the mechanism of membrane disordering and pore formation. Isothermal Titration Calorimetry (ITC) is used for assays such as demicellization, uptake-and-release and solubilization-and-reconstitution. Time-resolved DPH anisotropy and lifetime-based leakage assays are used to study membrane structural changes upon detergent incorporation in liposomes. Both lysoPC and DPC equilibrate with membranes very slowly. We hypothesize that the free energy penalty due to asymmetric membrane insertion limits the membrane uptake of lysoPC and DPC. This would be at variance to other detergents that induce membrane failure above a threshold asymmetry. Results are important for understanding mechanisms for membrane protein isolation and the interactions of amphiphilic biological compounds with lipid membranes.

422-Pos Board B191
Suppression of Cooperative Motions in Phospholipid Membranes by Osmotic Stress: Deuterium NMR Relaxation Study
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Understanding membrane dynamics is crucial to explaining the function of membrane proteins. Phospholipids are commonly employed as model systems to investigate biological membranes. The complex dynamic organization of phospholipid membranes spans several frequency decades, starting from subpicosecond local motions to millisecond collective dynamics [1]. Such motional frequencies can be accessed using various NMR relaxation methods. To address membrane dynamics mediated by osmotic stress, we measured ^{2}H longitudinal (R_{1Z}) and transverse quadrupolar echo (R_2^{QE}) relaxation rates for the liquid-crystalline phase of DMPC-d_{54} membrane bilayers. Osmotic stress was applied by both dehydration and osmolyte concentration [2]. The R_{1Z} values of individual acyl segments were independent of osmotic stress while the segmental order parameters (S_{CD}) and R_{1Z} profiles followed a theoretical square-law functional dependence [3]. The R_2^{QE} rates were found to be sensitive to osmotic pressure as well as the acyl position, thus yielding two important observations: enhanced transverse relaxation rates with increased amount of water per lipid, and limiting lower R_2^{QE} values as we dehydrate the membrane. The R_2^{QE} rates of the acyl segments and respective S_{CD} values tend to follow a square-law behavior [3] with increasing lipid dehydration. At higher hydration the square-law behavior is limited to those acyl segments deeper in the hydrophobic region, with a break as the head group is approached. These results clearly indicate that water enhances slow cooperative motions whereas they are suppressed by dehydration. Additional complementary Carr-Purcell-Meiboom-Gill (CPMG) dispersion measurements map the frequency dependence of relaxation rates. Such studies in presence of membrane proteins give insight into optimized lipid hydration for their biological functions.
[1] A. Leftin *et al.* (2011) *BBA* **1808**, 818-839.
[2] K.J. Mallikarjunaiah *et al*. (2011) *B.J*.**100**, 98-107.
[3] M.F. Brown (1982) *JCP***77**, 1576-1599.

423-Pos Board B192
Light Scattering on the Structural Characterization of DMPG Vesicles along the Bilayer Anomalous Phase Transition
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Highly charged vesicles of the saturated anionic lipid dimyristoyl phosphatidylglycerol (DMPG) in low ionic strength medium exhibit a very peculiar thermo-structural behavior. Along a wide gel-fluid transition region, DMPG dispersions display several anomalous characteristics, like low turbidity, high electrical conductivity and viscosity. Here, static and dynamic light scattering (SLS and DLS) were used to characterize DMPG vesicles at different temperatures. Similar experiments were performed with the largely studied zwitterionic lipid dimyristoyl phosphatidylcholine (DMPC). SLS and DLS data yielded similar dimensions for DMPC vesicles at all studied temperatures. However, for DMPG, along the gel-fluid transition region, SLS indicated a threefold increase in the vesicle radius of gyration, whereas the hydrodynamic radius, as obtained from DLS, increased 30% only. Despite the anomalous increase in the radius of gyration, DMPG lipid vesicles maintain isotropy, since no light depolarization was detected. Hence, SLS data are interpreted regarding the presence of isotropic vesicles along the DMPG anomalous transition, but highly perforated vesicles, with large holes. DLS/SLS discrepancy along the DMPG transition region is discussed in terms of the interpretation of the Einstein-Stokes relation for porous vesicles. Therefore, SLS data are shown to be much more appropriate for measuring porous vesicle dimensions than the vesicle diffusion coefficient. Although the underlying microscopic process which leads to the opening of pores in charged DMPG bilayer is very intriguing and deserves further investigation, one could envisage biotechnological applications, with vesicles being produced to enlarge and perforate in a chosen temperature and/or pH value, for a desired drug delivery process.

424-Pos Board B193
Membrane Structure and Intermembrane Forces Observed with Small Angle X-Ray Scattering
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Cellular functions rely on intermembrane interactions and forces that govern membrane structure and hence modulate lipid-protein interactions [1]. Moreover, the strengths of intermembrane forces vary with interlamellar distances. Here we address material properties of the membrane with structural deformation due to external stress using small-angle X-ray scattering (SAXS) spectroscopy. The SAXS technique has been extensively used to study membrane bilayers through application of osmotic pressure. However, distinguishing the effects of osmotic stress on intermembrane forces (separation force) and membrane deformation requires further investigation [2]. We subjected model membranes (DMPC) in the liquid-crystalline state to dehydration and high osmotic pressures (up to 25 MPa). The work of removal of water from the interlamellar region to the bulk water region restructures the membrane assembly and prompts us to examine membrane properties using complementary techniques. Using SAXS we were able to directly measure the interlamellar spacings and compare the results to solid-state ^{2}H NMR data [1,3]. We correlated the influences of dehydration and osmotic pressure in SAXS results through the interlamellar spacing. This approach allowed us to gauge the strength of intermembrane forces for a given hydration state. The combined techniques allowed us to estimate the area per lipid and structural deformation at the molecular level. Under high osmotic pressure or low hydration we found large area deformations up to 15% [1]. Temperature variation with this approach is used to discern entropic-based forces (lipid protrusions) and ordering-based forces (the hydration force). These findings show significant area deformation of membranes and provide insight into the forces that govern intermembrane interactions.
[1] K.J. Mallikarjunaiah *et al*. (2011) *B.J* **100**, 98-107.
[2] V.A. Parsegian *et al*. (1979) *PNAS* **76**, 2750-2754.
[3] H.I. Petrache and M.F. Brown (2007) *Meth. Mol. Biol*. **400**, 341-353.

425-Pos Board B194
Molecular Dynamics Simulation of Diacylglycerols in Phosphatidycholine Lipid Bilayers
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In this study, atomistic MD simulations were performed to investigate the interactions between diacylglycerols (i.e. DPG, POG, or DOG) and phosphatidylcholine (i.e. POPC or DOPC) bilayers. Our results show that diacylglycerols (DAG) increase acyl chain order, headgroup spacing and bilayer thickness, and reduce area-per-lipid. In a lipid bilayer, in order to avoid the unfavorable exposure of DAG hydrophobic parts to water, neighboring phospholipid (PC) headgroups move toward DAG to provide cover. This interaction between DAG and phospholipid is explained by the Umbrella Model. Comparing the three types of DAG in POPC and DOPC bilayers, DOG is located closer to the bilayer/aqueous interfaces than DPG and POG and it requires more coverage according to our umbrella index calculation, likely due to its longer and

unsaturated chains. The potentials of mean force were calculated for POPC in its bilayers containing DPG, POG, or DOG. Our results show that POPC flip-flop induces pores in pure PC bilayer, while adding diacylglycerol prevents the pore formation during flip-flop. System that contains POG has a higher free energy of desorption and lower free energy barriers for flip-flop, compared with systems that contain DOG or DPG. This indicates that POG is more favorable in POPC bilayer than the other DAGs, since its acyl chains are similar to POPC.

426-Pos Board B195

Local Micro-Partition Coefficients Govern Solute Permeability of Cholesterol-Containing Membranes

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It has long been recognized that the effects of both lipid structure and solute size on solute membrane permeability Pm are at odds with the solubility diffusion model, as both partition coefficient K and transverse diffusion coefficient D are assumed to be independent on solute penetration depth. As we show here, cholesterol may increase the apparent discrepancy between model and experiment, as Pm of our model solute tetraethylammonium (TEA) increased in its presence. Using scanning electrochemical microscopy we observed a cholesterol-induced increase in the steady state TEA flux across planar lipid bilayers from either phosphatidyl-choline or phosphatidyl-ethanomamine. The increase of cholesterol concentration above 20 mol % reversed the effect. According to molecular dynamics simulations, cholesterol did not take effect in altering D. At low concentrations cholesterol increased K in the region of the carbonyl groups, and at higher concentrations it decreased K in the tail region. Integrating over all penetration depth-dependent 1/(DK) values allowed prediction of Pm with reasonable accuracy at any cholesterol concentration.

427-Pos Board B196

Four-Component Phase Diagrams for DSPC/DOPC/POPC/CHOL and DSPC/DOPC/SOPC/CHOL Bilayer Mixtures

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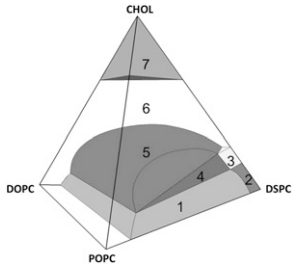
We report the phase diagram for the four-component DSPC/DOPC/POPC/Chol mixture and compare it to our recent findings for the DSPC/DOPC/SOPC/Chol mixture. Four-component mixtures allow exploring the transition from macroscopic-to-nanoscopic domains. We have found modulated phase morphology in a particular region of composition within the liquid-ordered (Lo) and liquid-disordered (Ld) coexistence region in DSPC/DOPC/POPC/Chol and DSPC/DOPC/SOPC/Chol mixtures. Overall, we conclude the following: (1) Phase diagrams are very similar with shifts observed in phase boundaries; (2) Comparing the phase behavior of the two mixtures, the striking difference is in the compositional location where modulated phases are seen: the SOPC-containing mixture requires much higher DOPC concentration to form modulated phases. This observation is consistent with lower line tension in the SOPC-containing mixtures as compared to the POPC-containing mixtures; (3) By controlling lipid composition, we observe distinct types of modulated liquid-liquid phase morphologies, including linear, irregular, and angular features in GUVs. These studies show that both the size and morphology of membrane rafts are controlled by the mixture composition and the type of low-melting lipid in mixtures with high-melting lipid and cholesterol.



428-Pos Board B197

SANS, FRET, and ESR Reveal <6nm Domains in Brain Sphingomyelin-Containing Membrane Models

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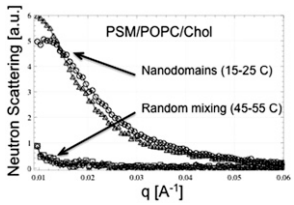
Membrane raft size measurements are crucial to understanding the stability and functionality of rafts in cells. The challenge of accurately measuring raft size is evidenced by the disparate nanometer-to-micron sizes of coexisting liquid domains that have been reported for the biologically relevant model membrane system SM (sphingomyelin)/POPC (1-palmitoyl-2-oleoyl-sn -glycero-3-phosphocholine)/Chol (cholesterol). By combining three techniques with different spatial sensitivities, electron spin resonance (ESR), Forster resonance energy transfer (FRET), and small-angle neutron scattering (SANS), we have significantly narrowed the uncertainty in domain size estimates for bSM (porcine brain SM)/POPC/Chol mixtures. Compositional trends in ESR and FRET data indicate domains, while SANS reports complete miscibility, consistent with the presence of domains no larger than approx. 6 nm radius at 25C in bSM/POPC/Chol. Upon replacing the natural SM mixture with synthetic palmitoyl SM, SANS reports coexisting liquid domains. As shown in the figure, PSM/POPC/Chol = 39/39/22 exhibits enhanced scattering indicative of coexisting domains at 15 and 25C (circles, triangles). Minimal scattering at high temperature (squares, diamonds) indicates nearly random mixing at 45 and 55C.



429-Pos Board B198

Towards a Better Raft Model: Modulated Phases in the 4-Component Bilayer Mixture, DSPC/DOPC/POPC/CHOL

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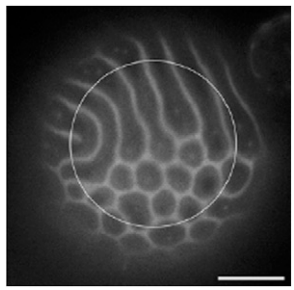
Liquid-liquid coexistence (Ld+Lo) within the DSPC/DOPC/POPC/CHOL mixture displays a nanoscopic-to-macroscopic transition of the phase domains as POPC is replaced by DOPC. Previously, we have shown that the nano-to-macro transition goes through a modulated phase regime, where patterned liquid-liquid phase morphologies were observed on giant unilamellar vesicles (GUVs). Here, we provide a more detailed investigation of the modulated phase regime along two different thermodynamic tielines within the Ld+Lo region of this 4-component mixture. Using fluorescence microscopy on GUVs, we found that modulated phases occur at relatively narrow DOPC/(DOPC+POPC) ratios along one tieline. This ''modulated phase window'' is greatly altered when cholesterol concentration is increased. Furthermore, when phase connectivity (percolation) changes, domain patterns also change. Monte Carlo simulations using a competing interactions model of line tension and curvature energies reproduced the patterns observed on GUVs. Sufficiently low line tension and sufficiently high bending moduli are required to generate stable modulated phases in the system examined. By tuning lipid composition, both domain size and morphology can be altered drastically within a narrow composition space. A possible mechanism for cells to reorganize plasma membrane compartmentalization is by tuning local membrane composition.



430-Pos Board B199

Effect of Cyclodextrin Structure upon Interaction of Cyclodextrin with Different Lipids Incorporated into Model Membrane Vesicles

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We have used methyl-beta- cyclodextrin (MbCD) to exchange membrane lipids between different vesicles in order to prepare model membrane vesicles with lipid asymmetry. To determine how to improve the efficiency of this method, the binding of lipids to various cyclodextrins (CDs) was investigated. The decrease in light scattering of multilamellar vesicles and the change in Förster resonance energy transfer (FRET) of labeled lipids incorporated into small unilamellar vesicles comprised primarily of unlabeled lipids were used to detect when vesicles are dissolved by binding of lipids to CD. A different FRET assay was used to detect when CDs catalyzed lipid exchange under conditions that vesicles do not dissolve. The effects of CD ring size (6, 7 or 8 sugar rings), substituants (hydroxy propyl, methyl, carboxymethyl and sulfate) and CD concentration were examined. It was found that while MbCD has the ability to dissolve lipid vesicles, most other CDs do not, although they are able to exchange lipids between vesicles. At lower MbCD concentrations, even MbCD loses the ability to dissolve vesicles, it retains the ability to exchange lipids. For MbCD, the concentration needed to dissolve about half of the vesicles was not strongly affected by lipid headgroup or acyl chain saturation, although there seemed to be some effect of acyl chain length.